Recent advances in cardiac regeneration: Stem cell, biomaterial and growth factors

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A B S T R A C T

Myocardial infarction has been reported to be responsible for about 7.3 million deaths each year globally. Present treatments for myocardial infarction have been more palliative rather than curative. Over the past few years, stem cells have demonstrated their potency in regenerating damaged cardiac tissue, especially after myocardial infarction. However, limited short half-life of the protein and cell therapy and low transplanted cell survival rate as demonstrated via several clinical trials have lead to development of more potent and novel delivery systems like biomaterial delivery system and the use of various growth factors. In this review, we will be enumerating and discussing the recent advances in cardiac regeneration with focus on stem cell, biomaterial and growth factors.

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1. Stem cells and growth factors application in cardiology

Various types of stem/progenitor cells have been commonly used to regenerate cardiac tissues damaged by myocardial infarction Table 1. Human stem cell-derived cardiomyocytes (hSC-DCMs) has been reported to be effective in regenerating cardiac tissue after myocardial infarction. There are four potential mechanisms have been proposed to be involved in stimulating myocardial repair and functional recovery: Firstly, cardiac regeneration: mesenchymal cells may have the tendency to differentiate into cells that looks like cardiomyocytes; Secondly, the cardiac repair might be through paracrine effect; Thirdly niche contribution: mesenchymal stem cell help to maintain the cardiac niche for cardiac stem cell; and lastly, because of its immunomodulatory tendencies, mesenchymal stem cells helps in the management of immune rejection and its also responsible for inflammatory regulation on cardiac repair and regeneration. It should be noted that all these proposed mechanisms work dependently to regulate stem cell function (Fig. 1) [1–4]. However, stem cell therapy has produced low cell survival and there are still major limitations encountered with consistent derivation of hSC-DCMs populations [6]. Several studies have reported the expression of cell inhibitors like p16 (INK), p21 and p19 (ARF) and cellular stress when mesenchymal stem cell (MSC) are cultured for a very long time, however co-culturing the MSC

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Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>Source</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonic stem cells</td>
<td>Inner cell mass of pre-</td>
<td>Pluripotent, self-renewal capacity</td>
<td>Graft versus host disease, ethical debate, and tumorgenesis</td>
</tr>
<tr>
<td>Mesenchymal stem cells</td>
<td>implantation blastocyst</td>
<td>Multipotent, easy to isolate and expand, lack of immunogenicity.</td>
<td>Heterogeneity</td>
</tr>
<tr>
<td>Endothelial progenitor cells</td>
<td>Bone marrow/peripheral blood</td>
<td>Movement from bone marrow or peripheral blood, important in neovascularization</td>
<td>Need for expansion, Heterogeneity, Electrophysiologically incompatible, lack of gap junction short survival, and limited supply</td>
</tr>
<tr>
<td>Skeletal myoblasts</td>
<td>Skeletal muscle</td>
<td>High scalability, resistance to ischemia, multipotent, no teratoma formation</td>
<td></td>
</tr>
<tr>
<td>Cardiac stem cells</td>
<td>Heart</td>
<td>Resident cells, robust cardiovascular differentiation potential, reduced tumor formation</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Mesenchymal mechanisms for cardiac repair. Through cardiac regeneration, paracrine mechanism, niche providing, and inflammatory control, MSCs can reduce infarct size, fibrosis, and myocardial remodeling, then increase angiogenesis and myocardial regeneration. Abbreviations: MSC, mesenchymal stem cells; CSC, cardiac stem cells; SC, stem cells; IV, intravenous; IC, intracoronary [5].

with vascular endothelial growth factor (VEGF) reduces the cellular stress and pro-survival factors like phosphorylated-Akt and Bcl-XL are increased.

Tang and other researchers reported that combined therapy of (MSCs + VEGF) to MI hearts leads to better cell engraftment and cardiac functioning compared to VEGF or MSCs therapy alone [7,8]. It should also be noted that insulin-like growth factor (IGF)-1 co-cultured with MSC is responsible for improving survival rate and also enhances the paracrine release of stromal cell-derived factor (SDF)-1α. Haider and co-worker also reported the role of insulin growth factor-1 (IGF-1) in facilitating the migration and differentiation of stem cells to injured heart [9]. As reported by Higashi and colleagues that IGF-1 is responsible for processes like development, cell growth and differentiation [10]. In addition some studies have demonstrated mesenchymal stem cells that overexpressed IGF-1/GF-1R used in an Intramyocardial transplantation procedure lead to enhanced and improved cardiac repair [11]. Furthermore, it has been reported that concurrent overexpression of Ang-1 and Akt in mesenchymal stem cells enhances the survival of these stem cells in the infarcted heart. It should also be noted that insulin growth factor and its receptor are widely distributed in cells like myocytes, cardiac progenitor cells (CPCs), and cardiac fibroblasts in the heart and that activation are responsible for so many biological activities including telomerase activity [12].

In addition to various activities coordinate by IGF-1, its also responsible for the releasing and expression of some growth factor like hepatocyte growth factor (HGF), basic fibroblast growth factor (b-FGF), and importantly, vascular endothelial growth factor which has been demonstrated by so many researchers as an essential regulator of the growth and development of new blood vessels in the heart under hypoxic condition [8]. VEGF has been reported to be indirectly secreted by MSCs and enhances regeneration of cardiac tissue after repairs [13]. At elevated levels, VEGF post-myocardial infarction have been demonstrated to be related with cardiovascular protection and improvements of clinical outcomes [14]. Recent clinical trials have also confirm the potency of VEGF in augmenting perfusion of ischemic myocardium in addition to decrease in defects [15,16]. In another experimental studies, it was reported that when differentiated human umbilical cord matrix stem cell combined with VEGF improved left ventricular dysfucntion, induces formation of new blood vessels and reduces fibrotic tissue formation in infarcted myocardium in eight weeks post MI when compared to the effect of VEGF alone [17].

Furthermore, functional studies have demonstrated that VEGF/MSC transplantation stimulates extensive angiogenesis and myogenesis via the increased expression of cardiac troponin T, CD31, and von Willebrand factor, in an injured heart as such leading to improved functioning of the left ventricle. Hatzistergos and co-worker reported that VEGF/MSC transplantation system enhances the process of angiogenesis through the process of SDF-1α pathway activation that in turn stimulates the differentiation of cardiac stem cells into endothelial cells in infarcted myocardium [18]. As stated before, VEGF is highly expressed by stem cells and MSCs. It should also be noted that MSC-conditioned medium evidently promotes the migration of cardiac stem cell (CSC) through the stromal cell derived factor (SDF) SDF-1α/CXCR4 pathway, which is proposed to be involved by the VEGF/VEGFR-1 and VEGFR-3 (vascular endothelial growth factor receptor-3) systems (Fig. 2).

In the past few years, clinical trials have been carried out using unfractionated adult bone marrow mononuclear cells (BMMNCs) because they are easy to aspirate from bone marrows, contains cardiomyocytes and endothelial precursor cells are present within the mononuclear cell fraction of bone marrow, easily injectable into the heart and lastly, they can be used when there are variable number of cells and different administration routes.
Briefly Table 2 gives a summary of clinical trials involving the use of BMMNCs and MSCs for cardiac repair. So many researchers have investigated the mechanism underlining the mesenchymal-based cell therapy for cardiac repair however more extensive work is still needed as cardiac cells are been replaced by fibrotic tissue [20]. Donor cells transplanted into myocardial infarcted area engraft into the recipient tissue to finally form into new, developing cardiomyocytes and this is the basis of myocardial cell transplantation. However most researchers do not subscribe to the notion that BMMNCs is the best stem cell source to be used for myocardial transplantation, recent differentiation advancements have identified human pluripotent stem cell(hPSC)- cardiomyocytes as a more potent and promising future of stem cell source, and they have been studied at preclinical level as regards their transplantation into the heart.

Maher and colleagues reported an improvement in the cardiac functions and survival of the animal model when hPSC-cardiomycocytes were engrafted into an infarcted region of several animal models [21]. Fernandes and co-worker further reported that hPSC-cardiomycocytes gives more stability and durable grafts in the infarcted heart when compared to BMMNCs and MSCs [22]. And more recently there has been positive studies proving the stability and the long-term cardiomyocyte engraftment and its functional incorporation in rodents have lead to the incorporation of this approach into a nonhuman primate injury model [23] like Pig- tailed macaques (Macaca nemestrina). In an experimental study reported by Chong and colleagues, the pig-tailed macaques received a reperfusion injury by inflating a balloon catheter into the distal left anterior descending coronary artery for 90 min followed by reperfusion (Fig. 3).

Table 2
Bone marrow-mesenchymal stem cell based clinical trial for cardiac repair.

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Numbers</th>
<th>Route</th>
<th>Delivery day after MI</th>
<th>Outcome</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM-MNCs</td>
<td>9–28 × 106</td>
<td>IC</td>
<td>7</td>
<td>Improved contractility and reduced infarct size</td>
<td>[24]</td>
</tr>
<tr>
<td>BM-MNcs</td>
<td>2.4 × 108</td>
<td>IC</td>
<td>3–7</td>
<td>Improved LV EF and reduced infarct size</td>
<td>[25]</td>
</tr>
<tr>
<td>BM-MNCs</td>
<td>24 × 109</td>
<td>IC</td>
<td>6</td>
<td>Improved EF and increased regional contractility</td>
<td>[26]</td>
</tr>
<tr>
<td>BM-MNCs</td>
<td>2.4 × 108</td>
<td>IC</td>
<td>4</td>
<td>Improved EF and reduced infarct size</td>
<td>[27]</td>
</tr>
<tr>
<td>BM-MNCs</td>
<td>11–90 × 106</td>
<td>IC</td>
<td>10–15</td>
<td>Significant functional improvement and reduced infarct size</td>
<td>[28]</td>
</tr>
<tr>
<td>BM-MNCs</td>
<td>3 × 108</td>
<td>IC</td>
<td>1</td>
<td>Decrease scar size but no improvement in LV EF</td>
<td>[29]</td>
</tr>
<tr>
<td>BM-MNCs</td>
<td>8.7 × 107</td>
<td>IC</td>
<td>5–8</td>
<td>No difference</td>
<td>[30]</td>
</tr>
<tr>
<td>BM-MNCs</td>
<td>13.4 × 107</td>
<td>IC</td>
<td>6–8</td>
<td>Improved LV function</td>
<td>[31]</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>48–68 × 1010</td>
<td>IC</td>
<td>18</td>
<td>Increased LV EF, regional contractility, and viability of infarct zone</td>
<td>[32]</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>2–4 × 106</td>
<td>IC</td>
<td>Directly</td>
<td>Reduced wall motion score index and improved myocardial viability and contractility</td>
<td>[33]</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>2.1–9.1 × 106</td>
<td>IC/IV</td>
<td>Directly</td>
<td>Perfusion defect and LV EF</td>
<td>[34]</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>0.5, 1.6, 5 × 106/kg</td>
<td>IV</td>
<td>1,2,3,6 months</td>
<td>Improved LV EF and reverse modeling</td>
<td>[35]</td>
</tr>
<tr>
<td>BM-MSCs</td>
<td>5–16 × 107</td>
<td>TESI</td>
<td>Directly</td>
<td>Cardiac remodeling, ESV and EDV, and regional contractility</td>
<td>[36]</td>
</tr>
<tr>
<td>CP-MSCs</td>
<td>6–12 × 108</td>
<td>EMG</td>
<td>Directly</td>
<td>LV EF and ESV and EDV</td>
<td>[37]</td>
</tr>
</tbody>
</table>

IC, intracoronary; IV, intravenous; BM-MNCs, unfractionated bone marrow mononuclear cells; MSCs, mesenchymal stem cells; CP MSCs, cardio-poietic mesenchymal stem cells; LV EF, left ventricular ejection fraction; EF, ejection fraction; TESI, transendocardial stem cell injection; EMG, electromechanical guidance; DI, direct intramyocardial injection.
2. Recent studies using biomaterial-based delivery systems in heart regeneration

Biomaterials have been demonstrated in four engineered different ways by which its abilities can be harnessed in the field of cardiology as shown in Fig. 4:

Several studies have demonstrated the potency of biomaterial-based delivery systems integrated with cell and protein therapies in improving the clinical outcomes of cells and proteins [38–44] in cardiac tissue regeneration. Presently several synthetic biomaterials that include caprolactone, polyglycolic and polyactic acids, polyurethane is under careful research investigation [45–53]. Table 3 gives a summary of their merits and demerits.

Leor and co-worker successfully implanted cardiac cell-seeded macroporous alginate scaffolds into an infarcted rat hearts [60]. Dar et al. Further corroborated Leor report and they reported that the seeded fetal rat cardiomyocytes were still viable within the scaffolds, in the space of 24 h [61]. They concluded that the cells successfully differentiated into mature myocardial fibers in the infarcted myocardium by observing the presence of cardiac muscle striation and gap junction formation. It should also be noted that angiogenesis was also observed subsequently leading to attenuation in left ventricular dilatation and improved heart function (Fig. 5) [60].

In pre-clinical animal models of MI, some researcher has reported the combination of cells or protein with biomaterials to
effective in improving cardiac functions after MI. Cardiac physiology and anatomy have been restored back to normal biological level by biomaterials together with the use of protein as drug delivery systems that afford protection for growth factors against in vivo degradation. In addition stem cells growth, survival and differentiation depends growth factors. Thus some researchers have researched extensively on the integration of stem cells, growth factors and biomaterial-based delivery systems and this integrated approach is known has tissue engineering triad (Fig. 6).

Fukuhara and co-worker first reported the use of a bioengineered nano-fibers scaffoldmade of polyglycolic acid succeeded in incorporating bone marrow stem cell and fibroblast growth factor. They finally concluded that the overall cardiac function and capillary density were significantly improved in BMSC-FGF-NFs treated animals when compared to BMSC or FGF loaded nano-fibers groups [63] and likewise, Diaz-Herráez and colleagues demonstrated the viability of using neuregulin (NRG)-releasing poly(lactic-co-glycolic acid-micro particles PLGA-MPs integrated with adult stem cell as a multiple growth factor delivery-based tissue engineering strategy for engraving into the infarcted myocardium [64]. They finally concluded that ADSC-NRG-MPs were effective and compatible with intramyocardial injection in a rat MI model and were still present 2 weeks after implantation proving long-term survival (Fig. 7).

Kraehenbuehl reported the delivery of thymosin β4 integrated with embryonic stem cells and smooth-muscle stem cells in ischemic injuries of a rat MI model. It should be noted that the thymosin Tβ4 protein protects cardiac muscle from death after ischemic damage and promotes angiogenesis by activating the survival kinase Akt, thereby announcing its importance as a cardiac regenerative molecule [65]. The use of self-assembling peptide RAD16-I has also been reported to be used to inject a hydrogels incorporating insulin-like growth factor integrated with cardiomyocytes [66] or cardiac progenitor cells [67] for cardiac repair. They finally concluded that in both administrations, the insulin-like growth factor significantly enhanced the recuperation of myocardial structure and function in rats one month after treatment. Furthermore PEGylated fibrin biomatrix has been
reported to competently bind to hepatocyte growth factor and capture bone marrow stem cell that lead to significant increase in cell frequency at myocardial infarction site at least for 4 weeks when compared to free cell administration [68]. In another study by Holladay and colleagues, mesenchymal stem cells were integrated with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide and N-hydroxysuccinimide together with IL-10(interleukin-10) and dendrimer polypelexes. They finally reported that after four weeks, they found stem cell retention and growth factors were significantly enhanced in animals which received MSC-i10-systems compared to the rest of the groups [69].

3. Conclusion

Over the past few years, cardiac regenerative medicine has made significant advancement [70–82]. Several pre-clinical studies have demonstrated and reported to be successful in the incorporation of cardiac patches to ischemia hearts and its potency to improve the ischemia heart contractile function. However more clinical studies using acellular biomaterials, bone marrow-derived cells and hPSC-derived cardiomyocytes should be carried out and challenges like stability, durability, and cell retention should be addressed using novel biomaterials and novel
techniques. In conclusion, the technical application of the tissue engineering triad approach on the integration of stem cells, growth factors and biomaterial-based delivery systems should be implemented.

Conflict of interest

None.

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Fig. 6. Tissue engineering triad.

Fig. 7. NRG-PLGA MPs. A) Scanning electron microscopy of NRG-PLGA MPs. B) Tissue retention of fluorescent PLGA MPs 1 month after intramyocardial injection in a rat MI model. C) Cardiac progenitor cell development 1 week after intramyocardial injection of NRG-PLGA MPs [64].


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